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Core Hypothesis Proposal form Revised April 29, 2002 Page 1

Initial Proposal for Core Hypothesis\Question
Please limit your response to 4 - 7 pages

I. *Proposed Core Hypothesis\Question*

In recent years it has been recognized that infection or inflammation appear to be associated with a surprising variety of disease conditions not considered previously to be of infectious origin, ranging from adult stroke and myocardial infarction to central nervous system disorders such as multiple sclerosis and Alzheimer's Disease (Grau 1997; Ridker et al 1997; Rothwell 1996). In this regard, pathogenetic links recently established ties between intrauterine infection/inflammation with preterm birth and other poor reproductive and neonatal outcomes. Furthermore, a small but growing body of evidence links these factors with poor long-term neurologic outcome, especially cerebral palsy (Grether and Nelson 1997; Nelson et al 1998; Yoon et al 2000). Prenatal exposure to infection has also been hypothesized in relation to schizophrenia (Brown and Susser 2002), autism (Hornig and Lipkin 2001), and cognitive impairment (Dammann et al 2002).

Characteristics of intrauterine infection as an adverse prenatal exposure have been assessed most extensively in relation to preterm birth (<37 wks). In preterm birth, intrauterine infection is typically caused by multiple types of vaginal bacteria of relatively low virulence; it ascends from the lower to the upper reproductive tract, first infecting the choriodecidual space, then the fetal membranes, the amniotic fluid and fetus itself; it may be chronic and is usually asymptomatic (Goldenberg et al, 2000). Pathogenesis is also likely to be complicated by genotypic characteristics of the mother or fetus affecting susceptibility to infection or the inflammatory response (Witkin et al 2000). It is likely that the impact of infection is proportionate to the level of fetal immaturity when it manifests.

The core hypothesis is that intrauterine exposure to infection/inflammation increases the risk of adverse pregnancy and neonatal outcomes as well as long-term sequelae (including cerebral palsy, autism, schizophrenia, and adult-onset cardiovascular and endocrinologic diseases) . Adverse pregnancy outcomes include preterm birth, abnormal fetal growth, coagulation defects, stillbirth, and birth asphyxia. Neonatal and long-term neurologic sequelae include cerebral palsy, periventricular leukomalacia, chronic lung disease, respiratory distress syndrome, necrotizing enterocolitis, autism, ADHD, schizophrenia, and mental retardation. Long term sequelae include adult-onset cardiovascular and endocrinologic diseases.

II. *Workgroup(s)*

Pregnancy and the Infant
Health Disparities and Environmental Justice
Early Origins of Adult Health
Early Pregnancy and Fertility

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IV. Public Health Significance

- *Prevalence/incidence*

The prevalence of exposure to intrauterine infection/inflammation varies depending on the type of exposure measurement, gestational age, physiologic compartment from which the sample is drawn, as well as sociodemographic factors such as race/ethnicity. Nevertheless, the exposure appears to be high. For example, as many as 70% to 80% of fetuses born at < 30 weeks' gestation and 28% of fetuses born at term after spontaneous labor may be exposed to chorioamnion colonization, while even 11% of term infants delivered by Cesarean section with intact membranes for maternal complications are also exposed (Hauth et al 1998). These estimates based on microbiologic culture techniques may actually be underestimates in comparison with those derived from more sensitive techniques, such as PCR (GonHalves et al 2002).

Thus, the characteristics of the proposed adverse intrauterine infectious/inflammatory exposure present numerous unsolved public health challenges related to detection of at-risk women and fetuses and design of specific and timely interventions. The high prevalence of the exposure and the significant burden of associated mortality and morbidity are compelling arguments in support of adopting intrauterine infection/inflammation as a core exposure of concern for the NCS.

- *Morbidity and Mortality*

In the United States, premature birth continues to be a leading contributor to perinatal morbidity and mortality with marked disparities of disease burden based upon race, ethnicity, and socioeconomic conditions. While accounting for approximately 11% of all births, premature births account for 60-80% of perinatal deaths (Goldenberg, 1998). Current clinical approaches have not decreased the rate of preterm birth in the last three decades (Goldenberg, 1998). Recently, in fact, there has been an increase in the incidence of births at <32 weeks' gestation (Hack et al 1995). Since the early 1980's, evidence has accumulated that a subsegment of preterm birth is likely to be caused by subclinical infection. It appears that very early preterm births (< 32 weeks' gestation) have the strongest association with intrauterine infection. For example, among births at 32 weeks or less, over 75% are accompanied by histopathologic evidence of inflammation in the placenta (Goldenberg, 2000; Goldenberg, 1998; Hack, 1995). Bacterial vaginosis is consistently associated with preterm birth across many populations (with relative risks of 2 to 8) (Gibbs, 1992).

Traditionally, clinicians think of clinically-evident complications of intrauterine infection such as maternal and neonatal sepsis, pneumonia, meningitis, and death. Enticing information indicates that additional complications of intrauterine exposure to bacteria results in periventricular leukomalacia (PVL), cerebral palsy (CP), bronchopulmonary dysplasia (BPD), respiratory distress syndrome (RDS), and other sequelae (Yoon 1997; Murphy, 1995; Grether, 1997; Alexander, 1998; Yoon 1997; Bejar R 1988; Perlman 1996). One hypothesis is that the fetus mounts an over-exuberant host response to in utero infection. In turn, this leads to preterm birth and fetal tissue damage likely mediated by the same cytokines and chemokines that are operative in the injury pathway with hypoxia. This condition has been referred to as the fetal inflammatory response syndrome (FIRS) (Gomez, 1998).

A number of studies that addressed the association between chorioamnionitis and cerebral palsy or cystic periventricular leukomalacia were included in a recently published meta-analysis (Wu 2000). Cerebral palsy is the most common neuromotor disability of childhood affecting 8,000 – 12,000 US newborns each year. Clinical chorioamnionitis was significantly associated with cerebral palsy and cystic periventricular leukomalacia in preterm infants, and cerebral palsy in term infants. (Appendix I)

A recent meta-analysis reported a summary relative risk for cerebral palsy of 1.9 (95% CI 1.5-2.5) associated with clinical chorioamnionitis in preterm infants and 4.7 (95% CI 1.3-16.2) associated with clinical chorioamnionitis in term infants (Wu 2002). Using a conservative estimate of the relative risk (lower end of the 95% confidence interval), the reviewers' calculations yielded an attributable risk of chorioamnionitis and cerebral palsy in premature infants of 28%. The association between chorioamnionitis and cerebral palsy appears to be stronger in term infants (who account for half of all cerebral palsy cases), although few studies have examined term infants and much work needs to be done to assess how intrauterine infection in term infants may be associated with perinatal depression, respiratory distress and neonatal encephalopathy. It has been suggested this may have the most immediate clinical implications (Nelson and Willoughby, 2002). Researchers have estimated that chorioamnionitis might account for 12% of all spastic cerebral palsy in term infants (Grether and Nelson 1997) and 28% of cerebral palsy on preterm infants (Wu and Colford, 2000). However, these estimates should be considered provisional until we greatly improve our understanding of the specific relevant exposures related to infection and inflammation and the relevant confounders. For example, the role of inflammatory cytokines as an independent etiologic factor for cerebral palsy or other neurologic sequelae has been stressed, yet antecedents of a cytokine response may be diverse (eg, infection, hypoxia-ischemia) and multiple antecedents may actually interact.

- *Perceived importance – how is answering this hypothesis going to improve the health and development of children.*

If a case can be made that antenatal infections/inflammatory exposure leads to long-term adverse outcome such as cerebral palsy it will result in a clinical paradigm shift – where the focus has been to prevent preterm delivery and the longer the baby is in utero the better – to a focus that a suboptimal intrauterine environment may in fact be more harmful than delivery – and more effort put toward preventing the initial cause (not the preterm labor alone).

The focus of the new strategies may be to monitor the uterine environment to insure that optimal conditions are maintained throughout pregnancy and develop mechanisms to prevent or treat infectious/inflammatory exposures and the adverse sequelae.

- *Preventability / Malleability*

Although there is considerable data associating intrauterine infections with preterm birth, the results of clinical trials of antibiotic treatment in humans have been largely disappointing. For example, when pregnant women with bacterial vaginosis and with previous preterm birth were treated prenatally, results have been mixed in terms of recurrence of preterm birth. In the setting of preterm premature rupture of the membranes, antibiotic treatment has resulted in a statistically significant delay in preterm delivery and a decrease in complications. However, the delay is relatively short, and newborn complications remain considerable (Mercer, 1995; Mercer, 1997; Kenyon, 2001). For women colonized genitally with Ureaplasma urealyticum or group B streptococci, antenatal treatment has not decreased preterm birth, low birth weight or pPROM (Eschenbach, 1991; Klebanoff, 1995). Further, empiric antibiotic administration in the setting of preterm labor with intact membranes does not prolong pregnancy (Romero, 1993; Egarter, 1996; Kenyon, 2001). Hence, a refinement in the fundamental concept of infection inflammation-induced preterm birth is required. Because the mechanisms responsible are complex, an intervention will most likely be beneficial only when there is a well-defined population at high risk for infection-induced adverse outcome. In addition, the antibiotic regimen (or other intervention) will need to be appropriately designed, including use of the proper course and timing. Effective intervention strategies require a better understanding of the intricate sequence

of microbiologic, inflammatory, and biochemical and clinical events inducing preterm birth, adverse neonatal and-long term neurologic sequelae.

V. Justification for a large, prospective, longitudinal study

The pathogenesis of intrauterine infection/inflammation, as well as each of the individual adverse reproductive and neurologic sequelae associated with it, is multifactorial. To more fully understand the multiple causal pathways leading to preterm delivery, cerebral palsy or other adverse sequelae, thereby identifying the independent causal role of intrauterine infection/inflammation in these outcomes, requires a prospective study in a large, diverse population. Our goals will be to identify specific infectious or inflammatory etiologic agents, their pathogenetic mechanisms and interrelations with each other, and their relations to other pre- and perinatal risk factors. To achieve these goals, multiple factors will need to be explored and at different time points in pregnancy and in newborns both to understand their ability to predict poor outcome and their relation to gestational age (ie critical pre- or perinatal times of exposure). Identifying critical exposure periods is of particular importance for the development of timely interventions and, given that the exposure may be chronic and is likely to be asymptomatic, prospective data collection is essential. Retrospective study designs relying on routine clinical data or biologic specimens will not address the research data needs in this area.

It is also essential to conduct the study in a large and diverse population. While the exposure may be common, the prevalence does vary by sociodemographic factors such as race/ethnicity. Furthermore, the outcomes of concern are of relatively low prevalence. For example, preterm births < 32 weeks are those most likely to be associated with infection yet only about 1% of all births occur at ≤32 weeks. Cerebral palsy is more rare, occurring in only 2-3/1000 births. In addition, since the pathogenetic relation between infection/inflammation and cerebral palsy may vary by gestational age, it is necessary to have sufficient study power to study the association in important analytic subgroups, eg spastic cerebral palsy in both term and preterm infants, not just sufficient study power estimated on the basis of all expected cases of cerebral palsy in the study population. Such considerations related to gestational age, or other important analytic subgroup parameters for the outcomes of concern, speak to the need for a large and diverse study population.

All sample sizes are based on an alpha of 0.05 and $\geq$ 80% power. We have included a number of the outcomes that will drive the sample size for this hypothesis. We have made the estimates of rates of exposure in the population based on current knowledge, however we appreciate that these estimates may be inaccurate. However, as noted in the final column, given a sample size of 100,000 pregnancies, we anticipate that all outcomes except that of cerebral palsy at term, will have significant power. For term cerebral palsy, a sample size of 100,000 pregnancies may be sufficient if the relative risk estimates are about 4.0 (rather than 2.0). This estimate of the magnitude of risk for cerebral palsy at term taken from the published meta-analysis results (Table 1) may be valid, but it is based on only 2 studies (Grether and Nelson 1998; Nelson and Ellenberg 1985) and the confidence limits of the published estimate were broad, 1.3-16.2. The outcomes selected, cerebral palsy (CP), neonatal encephalopathy, and preterm delivery were selected for the purposes of sample size needs due to their public health significance, relatively low prevalence, and the weight of evidence supporting an hypothesized association with intrauterine infection/inflammation. For all outcomes, it was thought necessary to compute sample size estimates for relevant gestational age groups. Furthermore, additional stratification by other important potential confounders, eg race/ethnicity, or multivariate adjustment would need to be considered for the analyses.

Outcome	Rate of exposure & Relative risk	Rate in exposed (per 1000)	Rate in unexposed (per 1000)	Total # needed (population)	Total number of pregnancies needed (estimates)
Cerebral palsy at term	10% exposure RR = 2	1	0.5	210,000 (term)	~233,333 (est 90% deliver >37wks and 1%stillbirth rate)
Cerebral palsy at term	10% exposure RR = 3	1.5	0.5	89,000 (term)	~100,000 (est 90% delivery > 37 wks and 1% stillbirth rate)
Cerebral palsy <32 weeks	40% exposure RR = 3	12	4	4,300 (<32 weeks)	~86,000 (est 5% deliver <32 wks and 1%stillbirth rate)
Moderate-severe neonatal encephalopathy (>34 weeks)	10% exposure RR = 2	3.5	1.5	60,000 (>34 wks)	~65,217 (est 93% deliver >34 wks and 1%stillbirth rate)

Power analysis

Power (in bold) for association between infection and preterm delivery <28 weeks as a function of rate of delivery ≤28 weeks (PTD<28), rate of infection in those ≤28 weeks (IR<28) versus >28 weeks (IR>28), and total sample size

Total sample size	PTD<28	IR<28 IR>28	3% 2%	3% 1%	4% 2%	5% 3%	7% 4%	70% 20%
100,000	1%		**52%**	**99%**	**96%**	**89%**	**99%**	**100%**
100,000	2%		**81%**	**100%**	**100%**	**99%**	**100%**	**100%**
80,000	1%		**44%**	**98%**	**91%**	**82%**	**96%**	**100%**
80,000	2%		**72%**	**100%**	**100%**	**98%**	**100%**	**100%**
60,000	1%		**35%**	**94%**	**82%**	**70%**	**89%**	**100%**
60,000	2%		**59%**	**100%**	**98%**	**94%**	**99%**	**100%**
60,000	3%		**76%**	**100%**	**100%**	**99%**	**100%**	**100%**
50,000	1%		**30%**	**89%**	**74%**	**62%**	**83%**	**100%**

The estimates used in the above table for infection in preterm delivery < 28 weeks and controls are very conservative. Sample size of 60,000 will have sufficient power in most of these conservative estimates. Sample sizes >60,000 or rate of delivery <28 weeks of >2% will have sufficient power in all estimates. Using a higher gestational age limit for preterm delivery (e.g. < 32 weeks) will yield larger proportion of cases and higher estimates of power for any scenario compared with those shown above for PTD <28 weeks.

Scientific Merit

Support for the core hypothesis is based upon considerable research although the strength of the evidence varies among the end points studied. For example, the strength of the relationship between intrauterine infection and preterm birth is supported by extensive epidemiologic, microbiologic and clinical data (Gibbs RS, Subclinical infection as a cause of premature labor. Chapter 19 in Sweet RL and Gibbs RS, Infectious Diseases of the Female Genital Tract, 4th edition, 2002, Lippincott Williams and Wilkins, Philadelphia, PA). These data may be summarized as follows:

1) Histologic chorioamnionitis is increased in patients who have preterm birth;
2) Clinical infection is increased in both mothers and infants after preterm birth;
3) There are significant associations of some lower genital tract organism/infections with preterm birth or preterm premature rupture of membranes. There is also evidence that infections remote

from the genital tract also lead to prematurity. One area of active investigation involves study of maternal periodontitis and prematurity (Offenbacher 2001)

4) There are positive cultures of amniotic fluid or membranes from some patients who have preterm labor or preterm birth;
5) There are clear markers for infection in patients who had preterm birth;
6) Selected bacteria or their products are able to induce preterm birth in experimental animal models;
7) Some antibiotic trials have shown a lowering of the rate of preterm birth or have deferred preterm birth.

Support for the relationship between intrauterine exposure to infection/ inflammation and cerebral palsy or cystic periventricular leukomalacia is more limited, but compelling. Experimental intrauterine infection in rabbits leads to brain white matter lesions (Yoon 1997). In preterm infants, Yoon et al reported a four- to six-fold increased risk of brain white matter damage associated with elevated interleukin 1β (IL-1β) and interleukin 6 (IL-6) levels in amniotic fluid sampled within three days of delivery; a six-fold increased risk of brain white matter damage associated with elevated IL-6 levels in umbilical cord blood; and a six-fold increased risk of CP associated with elevated amniotic fluid levels of IL-6, interleukin-8 (IL-8), and matrix metalloproteinase-8 (an enzyme produced by neutrophils of fetal origin and significantly higher in the amniotic fluid of infants with funisitis than in infants without funisitis). These estimates of risk of both brain white matter damage and CP from elevated cytokine levels were obtained after adjusting for gestational age. However, Nelson et al examined cytokine levels in blood obtained from archived dried blood spots (retained after screening for congenital metabolic disorders) of preterm newborns and found no differences in levels of 25 analytes, including IL-1, IL-6, and tumor necrosis factor-α (TNF-α) between children with and without CP; gestational ages of the two groups of children were similar.

Nelson et al explored the association between approximately 50 markers of inflammation, autoimmune, and coagulation disorders measured in archived newborn dried blood spots and spastic CP in a sample of 31 predominantly term children with CP and found higher concentrations of cytokines IL-1, IL-8, interleukin 9 (IL-9), TNF-α, and RANTES (regulated on activation, normal T-cell expressed and secreted) in children with CP than in control. The sensitivity and specificity of these markers for CP were 100%. Children with CP also had higher concentrations of other cytokines, chemokines, other markers of inflammation, and markers of coagulation abnormalities than did control children; for some of these other markers the sensitivity and specificity for CP exceeded 88%. In a second study based on the same sample of 31 children, Grether et al reported higher concentrations of interferons-α, -β, and -γ in children with spastic CP relative to control children. Nelson et al[37] found that analyte levels were not related to day of life in which the blood was drawn, suggesting that cytokine overexpression may be sustained in term infants diagnosed with CP and related to exposure to chronic inflammation originating before birth.

The extensive epidemiologic data of cerebral palsy or cystic periventricular leukomalacia and chorioamnionitis has previously been summarized. These are intended as illustrative examples of the scientific base. For other end points such as schizophrenia, autism, and cognitive impairment, the data are less well-developed.

- *How will answering this hypothesis/question advance our understanding*

Despite the strong epidemiologic association and the intriguing biochemical/cytokine data, critical pieces of the puzzle are missing. One such piece of evidence is markers for populations at risk, i.e., patients likely to suffer adverse pregnancy outcomes after exposure to intrauterine bacteria. Another gap in our knowledge is the mechanism by which intrauterine exposure to bacteria leads to these adverse sequelae.

One widely discussed mechanism is that bacteria ascend into the uterine cavity or infect the cavity by a blood-borne route. An alternate hypothesis is that a chronic low-grade endometritis that precedes the pregnancy itself leads to either premature rupture of membranes, premature labor or both. Infection/inflammation by either route, in turn, triggers a fetal or maternal production of inflammatory cytokines via macrophage activation, and these then stimulates prostaglandin synthesis leading to preterm birth. An over exuberant response of cytokines in fetal tissues is then reported to lead to fetal tissue damage such as in the lung, brain, and gut.

Answering this question/hypothesis will provide information on the population(s) at risk for preterm birth and other developmental sequelae. We will also gain important information on the mechanism of infection/inflammation-induced disease outcomes only then can rational therapeutic trials be designed and launched.

Currently, antibiotics are extensively used to prevent preterm birth and/or clinically evident infection. Up to 25% of patients may receive antibiotics during labor to prevent perinatal Group B streptococcal sepsis, and it is a widely used practice to administer a combination of antibiotics to patients with preterm premature rupture of the membranes. Answering this question/hypothesis may lead to methods to avoid unnecessary use of antibiotics as well.

VI. Potential for innovative research

The association between maternal infection and cerebral palsy generates a number of important and, as yet, unanswered etiologic questions, and also has important clinical implications. What are the dangers of occult infection, especially in premature labor or premature rupture of the membranes, and its consequences when delivery is delayed? What is the role of antenatal or intrapartum antibiotic therapy in preventing cerebral palsy? What are the risks when aiming for vaginal delivery in pregnancies complicated by chorioamnionitis? What are the environmental or genetic factors that may predispose or decrease the threshold for infection-associated neurologic injury? When is the critical time during pregnancy when the neurologic effects of intrauterine infection/inflammation arise – and how may that differ between the effects observed in preterm infants and term infants? What are the specific infectious or inflammatory etiologic agents, their pathogenetic mechanisms and interrelations with each other, and their relations with other pre- and perinatal risk factors? Multiple factors need to be explored and at different time points in pregnancy both to understand their ability to predict poor outcome and their relation to gestational age. All these additional questions may be answered using data collected for the proposed study.

Some women are at higher risk for preterm birth. African-American women, for example, have higher rates of preterm birth and low birth weight infants and are also more likely to have bacterial vaginosis, histologic chorioamnionitis, and clinically evident intrauterine infection in late pregnancy or in the puerperium (Goldenberg, 2000; Hillier,1995; Smulian, 1999; Goldenberg, 1996; Hillier, 1988). A genetic mechanism in the control of immune response has been proposed (Mira, 1999; Calvano,1996). Among African-American women with pPROM, carriers of the rarer allele of the polymorphism at the position -308 in the gene for TNF-alpha were more common in those who delivered preterm than among controls ($p=0.008$) (Roberts, 1999). Thus, some groups appear to have a hereditary predisposition to up-regulation of inflammatory cytokines. In response to intrauterine infections, these women may mount an over-exuberant cytokine response (Roberts, 1999).

Thus, genetic identification of patients at risk could well be incorporated into this longitudinal study. Large-scale screening for single nucleotide polymorphism can be performed using DNA microarray technology applied to parental and neonatal tissue. The association between infection and adverse outcomes is most likely multifactorial and not dependent on a single polymorphism. With the proposed

analysis, a certain profile comprised of multiple polymorphisms may be found to be the central link between infection and adverse outcomes.

The proposed study incorporates numerous tests of fetal and maternal condition (including the genetic testing). Algorithms for prediction of preterm labor can be devised by applying "Neural Networks" to the data obtained. Such a method may provide more accurate prediction of preterm delivery than previous methods using single variables. The same methods can also be used to determine which fetus is at risk for adverse outcomes. Such an analysis will have a major impact on management of pregnancies. As an example, tocolysis may be withheld from a patient at high risk for infectious related maternal or perinatal morbidity. A large, prospectively-collected dataset with multiple variables is required for these analyses.

It has been postulated that suboptimal fetal growth may be associated with preterm birth. Such a deviation in fetal growth was best detected using individualized growth assessment rather than comparison to population nomograms. The proposed study will allow us to determine parameters that determine fetal growth in normal environment, and in turn devise the formula to calculate individualized growth potentials. This can then be used to test several hypothesis relating to intrauterine environment and adverse outcomes including preterm birth, as well as adult-onset diseases.

While the duration of pregnancy is measured in days, the consequences of the in utero exposure have far reaching implications. The importance of capturing key information and data from this time period cannot be overemphasized as this information has over arching implications for all other aspects of the National Children's Study. By virtue of the need to collect extensive information on the woman during pregnancy and to collect substantial numbers of biologic specimens which will be placed into a repository, countless other potential areas of interest will be amenable to investigation in the future, e.g., cardio vascular disease and stroke or metabolic diseases such as diabetes mellitus. Biologic specimens will be available that can be analyzed for key markers to include infectious agents, biochemical markers of stress, mediators of disease processes such as cytokines and chemokines, gene expression, and combinations and interactions of all of the above.

Yet, another opportunity for innovative research lies in the application of new diagnostic technologies. Traditional methods to detect infection have used isolation of microbes or detection of host humeral or cellular responses. However, these methods may be insensitive, cumbersome, and expensive. By establishing a repository of critical specimens, we will be able to employ current molecular methods of diagnosis (such as PCR and LCR) as well as even more sensitive methods likely to be developed over the course of the study.

The above is but a brief overview and one example of the potential for innovative research in this area. It is again emphasized that the data and tissue repository acquired during pregnancy may be of incalculable value to every area of the National Children's Study and is an opportunity that if missed, cannot be recovered.

- *Feasibility*

As previously addressed in Section 5, this study with a projected enrollment of 100,000 will be sufficiently powered to look at end points of cerebral palsy in births occurring at <32 weeks and moderate or severe neonatal encephalopathy at >34 weeks gestational age births. The study may or may not have sufficient power to investigate term cerebral palsy, depending on the estimated strength of the association. As regards the critical period for exposure and outcomes, depending upon the specific outcome, the critical exposure may occur at different times throughout pregnancy. Because the majority of these outcomes can only be identified after birth and in the neonate, infancy, pre-school or school aged children, it will be necessary to collect data on all women enrolled in the study. A standardized collection

instrument will be developed after input from all other working groups inasmuch as it will be critically important to capture pregnancy data of interest to other working groups (in addition to our own).

Because the disease processes of interest cannot be identified until the conclusion of pregnancy, and in many instances well beyond, it will be necessary to collect and store many specimens for subsequent analysis. Specimens will only be acquired in conjunction with regularly scheduled tests that fit within the framework of obstetric standard of care. Women will not undergo any invasive procedures simply for specimen collection. Specifically, specimens will be collected consistent with the existing standards of care and augmented as specifically indicated by occurrences such as infections during the course of pregnancy. By way of example, if an amniocentesis is done for genetic purposes or diagnostic purposes a portion of the amniotic fluid will be stored for future analysis by either currently existing technologies, e.g., PCR or technologies that might be developed in the future. As a minimum we will attempt to collect and store maternal serum from each trimester of pregnancy and from any episodes of acute illness felt to be infectious in nature. We will additionally collect and store cord blood at the time of birth. Placenta and umbilical cord will also be collected and frozen "fresh" as well as fixing this tissue and retaining paraffin blocks of tissue in the repository. In instances when a cordocentesis or amniocentesis is performed for clinical indications any excess amounts of these specimens beyond those necessary for clinical diagnostics will be stored. Additionally, vaginal/and cervical swabs will be collected at the new OB visit and at delivery and preserved for subsequent analysis. The approach of collecting specimens in each woman and placing them in a repository will allow maximum capture of the disease processes of interest and minimize cost of analysis inasmuch as appropriate controls can be selected from the overall population for virtually any disease process which one determines to be of interest.

Similar to any pregnancy, contact with the woman would be intensive throughout the antepartum period and during their admission for birth or for any medical or surgical complications during the pregnancy. Contact would also be intensive during the newborn period and for neonatal high risk follow-up. Developmental follow-up would be scheduled at selected points in the postnatal period; for example, at age 12 months, 36 months, and 6 years. Routine physical and neurodevelopmental parameters would be measured in the children at each of these encounters.

Because the contacts with these patients will essentially fall within the scope of standard of care during the pregnancy, additional cost will be relatively minor and entail until simply maintaining the pregnancy and infant/childhood data-base and the tissue sample repositories. Subsequent costs of analysis will be dependant upon the specific tests to be conducted. Additional burdens placed on the family are judged to be modest overall. Although the postnatal evaluations may be time consuming they would be scheduled relatively infrequently. Ethical issues are judged to be modest to minimal and this is especially the case if confidentiality of the data base and testing information is assured to the patient.

References

Barker DJP. Mothers, babies and health in later life, 2nd Ed. Churchill Livingstone, London, 1998;2 Ed.

Nathanielsz PW. Life in the Womb: The origin of health and disease. Promethean Press, Ithaca, 1999.

Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. New England J Med 2000;18;342(20):150-7

Hillier SL, Nugent RP, Eschenbach DA, et al. The association of bacterial vaginosis, bacteroides, and mycoplasma hominis with preterm low birth weight. New England J Med 1995;333:1737-42

Smulian JC, Shen-Schwarz S. Vintzileos AM, Lake MF, Ananth CV. Clinical chorioamnionitis and histologic placental inflammation. Obstet Gynecol 1999;94(6):1000-5.

Goldenberg RL, Thom E, Moawad AH, Johnson F, Roberts J, Caritis SN, et al. The preterm prediction study: fetal fibronectin, bacterial vaginosis, and peripartum infection. Obstet Gynecol 1996;87:656-60.

Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. New England J Med 1988;319:972-8.

Mira JP, Cariou A, Grall F, Declaux C, Losser MR, Heshmati F, Cheval C, Monghi M, Teboul JL, Riche F, Leleu G, Arbibe L, Mignon A, Delpech M, Dhainaut JF. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 1999;11;282(6):561-8

Calvano SE, van der Poll T, Coyle S, Barie P, Moldawer L, Lowry S. Monocyte tumor necrosis factor receptor levels as a predictor of risk in human sepsis. Arch Surg 1996;131:434-7.

Roberts AK, Monzon-Bordonaba F, Van Deerlin PG, Holder J, Macones GA, Morgan MA, Strauss JF III, Parry S. Am J Obstet Gynecol 1999;180 (5):1297-302.

Yoon BH, Jun JK, Romero R, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1β, and tumor necrosis factor-α), neonatal brain white matter lesions, and cerebral palsy. *Am J Obstet Gynecol*. 1997;177:19-26.

Yoon BH, Romero R, Yang SH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. *Am J Obstet Gynecol*. 1996;174:1433-1440.

Yoon BH, Park JS, Romero R, et al. Intraamniotic inflammation and the development of cerebral palsy at three years of age. *Am J Obstet Gynecol*. 1999;180:S2.

Yoon BH, Romero R, Kim M, et al. Elevated amniotic fluid matrix metalloproteinase-8 and the subsequent development of cerebral palsy. *Am J Obstet Gynecol*. 2001;184: S3.

Yoon BH, Oh SY, Kim M, Jongkwan J, Romero R. Amniotic fluid matrix metalloproteinase-8: An index of funisitis and the fetal inflammatory response syndrome. *Am J Obstet Gynecol*. 2001;184: S31.

Nelson KB, Grether JK, Dambrosia JM, Dickens B, Phillips TM. Cytokine concentrations of neonatal blood of preterm children with cerebral palsy (CP). *Am J Obstet Gynecol*. 2000;182:S47.

Nelson KB, Dambrosia JM, Grether JK, Phillips TM. Neonatal cytokines and coagulation factors in children with cerebral palsy. *Ann Neurol*. 1998;44:665-675.

Grether JK, Nelson KB, Dambrosia JM, Phillips TM. Interferons and cerebral palsy. *J Pediatr*. 1999;134:324-334.

Offenbacher S, Lieff S, Boggess KA et al. Maternal periodontitis and prematurity. Part I: Obstetric outcome of prematurity and growth restriction. Annals of Periodontology 2001; 6:164-174

Yoon BH, Kim CJ, Romero R et al , Experimentally induced intrauterine infection causes fetal brain white matter lesions in rabbits, American Journal of Obstetrics and Gynecology 1997; 177:797-802

Bejar R, Wozniak P, Allard M, Benirschke K, Vaucher Y, Coen R, Berry C, Schragg P, Villegas I, Resnik R. Antenatal origin of neurologic damage in newborn infants. I. Preterm infants. Am J Obstet Gynecol 1988;159:357-63

Perlman JM, Risser R, Broyles RS. Bilateral cystic periventricular leukomalacia in the premature infant: associated risk factors. Pediatrics 1996;97:822-7

Wu YW, Colford JM. Chorioamnionitis as a risk factor for cerebral palsy: a meta-analysis. JAMA 2000;284:1417-24

Appendix I: Number of studies analyzed and summary risks of cerebral palsy (CP) or cystic periventricular leukomalacia (cPVL) in preterm and term infants according to classification of chrorioamnionitis (data from ref. 229).Wu and Colford, 2000).

	Number of Studies	Relative Risk	95% Confidence Interval
Preterm Delivery			
Clinical chorioamnionitis and CP	11	1.9	1.4 – 2.5
Clinical chorioamnionitis and cPVL	6	3	2.2 – 4
Histologic chorioamnionitis and CP	5	1.6	0.9 – 2.7
Histologic chorioamnionitis and cPVL	2	2.1	1.5 – 2.9
Any chorioamnionitis and CP	12	1.8	1.5 – 2.3
Term Delivery			
Clinical chorioamnionitis and CP	2	4.7	1.3 – 16.2
Histologic chorioamnionitis and CP	1	8.9	1.9 - 40
All Gestational Ages			
Clinical chorioamnionitis and CP	3	2.1	1.5 - 3